AD-A124 555 THE MUTAGENIC POTENTIAL OF (E)-1234-TETRAHYDRO-6-METHYL-1-(2-METHYL-1-OXO..(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA L J SAUERS ET AL. NOV 82 F/G 6/20 1/1

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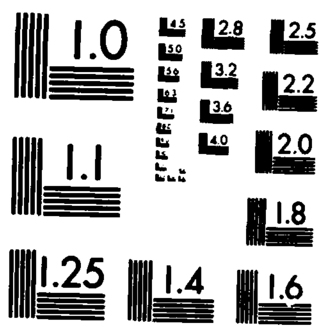

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

INSTITUTE REPORT NO. 136

THE MUTAGENIC POTENTIAL OF: (E)—1,2,3,4—tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5)

LEONARD J. SAUERS, BA, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1982

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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29 Nov 1982

(Signature and date)

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER LAIR Institute Report No. 136	2. GOVT ACCESSION NO. AD-A124555	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) The Mutagenic Potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5)		5. TYPE OF REPORT & PERIOD COVERED Final 21 April - 21 May 1982
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Leonard J. Sauers, BA, SP5 John T. Fruin, DVM, PhD, COL VC		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, MD 21201 & Tox Grp, Div of Rsch Spt, LAIR, Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Project 3M162779A871
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE November 1982
		13. NUMBER OF PAGES 29
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Mutagenicity, Toxicology, Ames Assay, (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) was assesed by using the Ames salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 10^{-1} mg/plate to 3.2 x 10^{-5} mg/plate. It was determined that the test substance did not have mutagenic potential.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The mutagenic potential of (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 10^{-1} mg/plate to 3.2×10^{-5} mg/plate. It was determined that the test substance did not have mutagenic potential.

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Same as above

PROJECT: 3M162779A871, Development of Repellents Against Medically Important Arthropods, WU 201, APC TL01

GLP STUDY NUMBER: 82012

STUDY DIRECTOR: COL John T. Fruin, D.V.M., PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, BA

RAW DATA AND DATA MANAGEMENT: A copy of the final report, retired SOPs, raw data, and chemical, analytical, stability, and purity data of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5)

INCLUSIVE STUDY DATES: 21 April - 21 May 1982

OBJECTIVE: To determine the mutagenic potential of the above compound using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The plate incorporation method was followed. The test substance was dissolved in ethanol and this diluent was checked for sterility.

ACKNOWLEDGMENTS

The authors wish to thank PFC Paul Mauk, BS; Carolyn Lewis, MS; and John Dacey for their assistance in performing the research.

Signatures of Principal Scientists involved in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Leonard J. Sauers 17 Oct 82

LEONARD J. SAUERS, BA/DATE
SP5
Principal Investigator

John T. Fruin 19 Oct 82

JOHN T. FRUIN, DVM, PhD/DATE
COL, VC
Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 9 Aug 82

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study #82012 the following inspections were made:

21 Apr 82
23 Apr 82

The report and raw data for this study were audited on 5 Aug 82.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 7 July 82 report to management and the Study Director.

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

TABLE OF CONTENTS

THE MUTAGENIC POTENTIAL OF: (E)–1,2,3,4–tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline (CHR 5) – Sauers and Fruin

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsomal enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon`s mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of *Salmonella typhimurium* that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the archives of the Quality Assurance Unit.

In this series of tests for the detection of mutagenic potential of different agents, we compare the spontaneous reversion values with our own historical values and these cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs). A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance are added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined by the supplier, and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen in the Salmonella/Mamallian Microsome Mutagenicity T--t: Assay of over 300 Chemicals," have concurred on the test's abil..y to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames (2). He assumed that a compound which causes twice the spontaneous reversion rate and a correlated dose response is mutagenic

Chemical Analysis

Our information on the chemical analysis of CHR5 was obtained from Starks Associates (Appendix A).

RESULTS AND DISCUSSION

Throughout this report, (E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline will be referred to by its respective code name, CHR 5.

On 21 April 1982, the toxicity level determination was performed on the test compound. All sterility, strain verification and negative controls were normal (Table 1). A toxic response was observed at the highest dose used; therefore, 10^{-1} mg/plate was designated as the initial dose for the assay (Table 2).

On 23 April 1982, the Ames Assay was performed on CHR 5. All strain verification and sterility controls were normal for this experiment (Table 3). Expected results were obtained for all positive and negative controls (Table 4). The bacterial strains were exposed to doses ranging from 10^{-1} mg/plate to 3.2×10^{-5} mg/plate of test substance. In no case was a dose response or a doubling of the spontaneous reversion rate observed (Table 5).

CHR 5 was tested previously (LAIR Institute Report 109). At that time, a solution of an unknown concentration was assayed. The Ames test was repeated due to the new lot and controlled concentration.

CONCLUSION

Based on the Ames Assay, CHR 5 is not mutagenic at the levels tested.

RECOMMENDATION

We recommend that candidate insect repellent CHR 5 be tested further with other toxicological assays if efficacy tests show this compound to be a promising repellent.

REFERENCES

1. McCANN, J., E. CHOI, E. YAMASAKI, and B. N. AMES. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 72:5135-5139, 1975

2. AMES, B. N., J. McCANN and E. YAMASAKI. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 31: 347-364, 1975

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 15 February 1982

4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties, J Biol Chem, 218: 97-106, 1956

5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976

Chemical Analysis

APPENDIX A

DATA SHEET FOR COMPOUNDS

WR NO.	SUBMITTER	SUBMITTER KEY NR.
WR250018AA	Starks Associates, Inc. 1280 Niagara Street Buffalo, New York 14213	0205

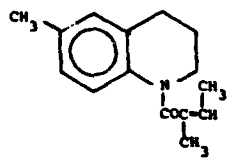
DATE SHIPPED	DAY	MO	YR	DATE RECEIVED	DAY	MO	YR	DATE ACKNOWLEDGED	DAY	MO	YR
	15	2	82		17	2	82				

NAME OF COMPOUND: (E)-1,2,3,4-Tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl)quinoline 0530

STRUCTURE

ST E

2.5



BK16177

MOL. FORMULA	MOL. WT
$C_{15}H_{19}NO$	229.324

APPEARANCE: white crystalline solid

QUANTITY	CODE NR.
1061 g	JF15-22-2

NOTEBOOK REF.	PREPARED BY
JF15-22-2	J. F. Novotny

TEST SYSTEM

B.P.	M.P.	REFRACT. INDEX
	37-37.5°	

attached spectrum

attached spectrum

ANALYSES

ELEMENT	CALCULATED	FOUND	WR FOUND
C	78.56	78.90	
H	8.35	8.52	
N	6.11	6.00	
S			

CHROMATOGRAPHY* Homogeneous (Baker precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60 F-254; detection - ultraviolet light).

LITERATURE
1. The compound is unknown to the chemical literature.

STABILITY (Check Where Applicable)

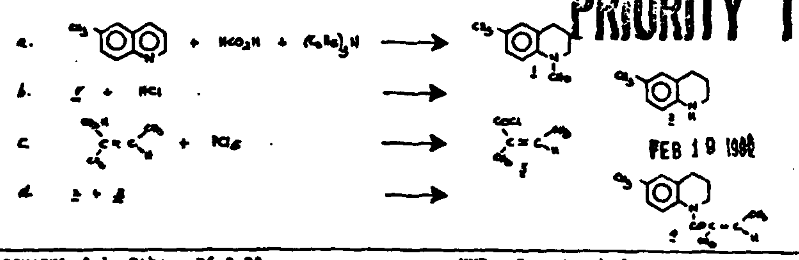
	STABLE	UNSTABLE	OTHER	STABLE	UNSTABLE
ACID	☐ 56	☐	______	☐ 60	☐
BASE	☐ 57	☐	______	☐ 61	☐
HEAT	☐ 58	☐	______	☐ 62	☐
LIGHT	☐ 59	☐			
	1	2		1	2

HYGROSCOPIC YES ☐ NO ☒ 63

SOLUBILITY (Check Where Applicable)

	V. SOL	MOD. SOL	IN SOL		V. SOL	MOD. SOL	IN SOL
WATER 64	☐	☐	☐	CHLOROFORM 70	☒	☐	☐
ACID 65	☐	☐	☐	ETHER 71	☐	☒	☐
BASE 66	☐	☐	☐	PET. ETHER 72	☐	☐	☐
METHANOL 67	☐	☐	☐	BENZENE 73	☐	☐	☐
ETHANOL 68	☐	☐	☐	______ 74	☐	☐	☐
ACETONE 69	☒	☐	☐	______ 75	☐	☐	☐
	1	2	3		1	2	3

EQUATIONS INDICATING SYNTHETIC ROUTE



PRIORITY 1

FEB 19 1982

REMARKS: * 1. Ether; Rf=0.83
2. Ethyl acetate; Rf=0.64
3. Methylene chloride; Rf=0.30, elongated spot

NMR - See attached spectrum
Solvent of recrystallization - ether

COMMERCIALLY DESCREET ☐	SYNTHESIZED UNDER GOV'T SUPPORT ☒	GIFT ☐	PURCHASED ☐

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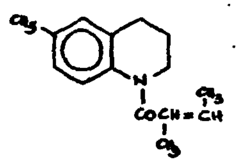
JF 15-22-2



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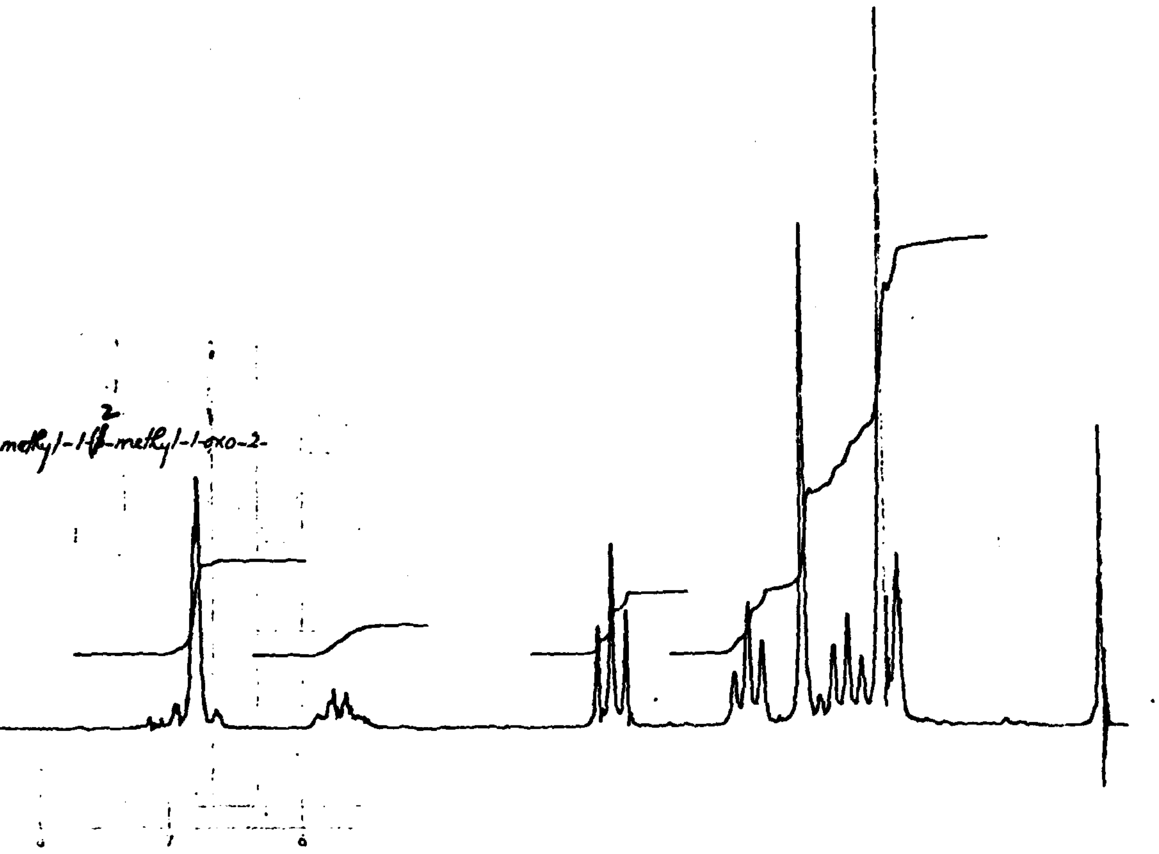

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SWEEP TIME
SWEEP WIDTH
END OF SWEEP

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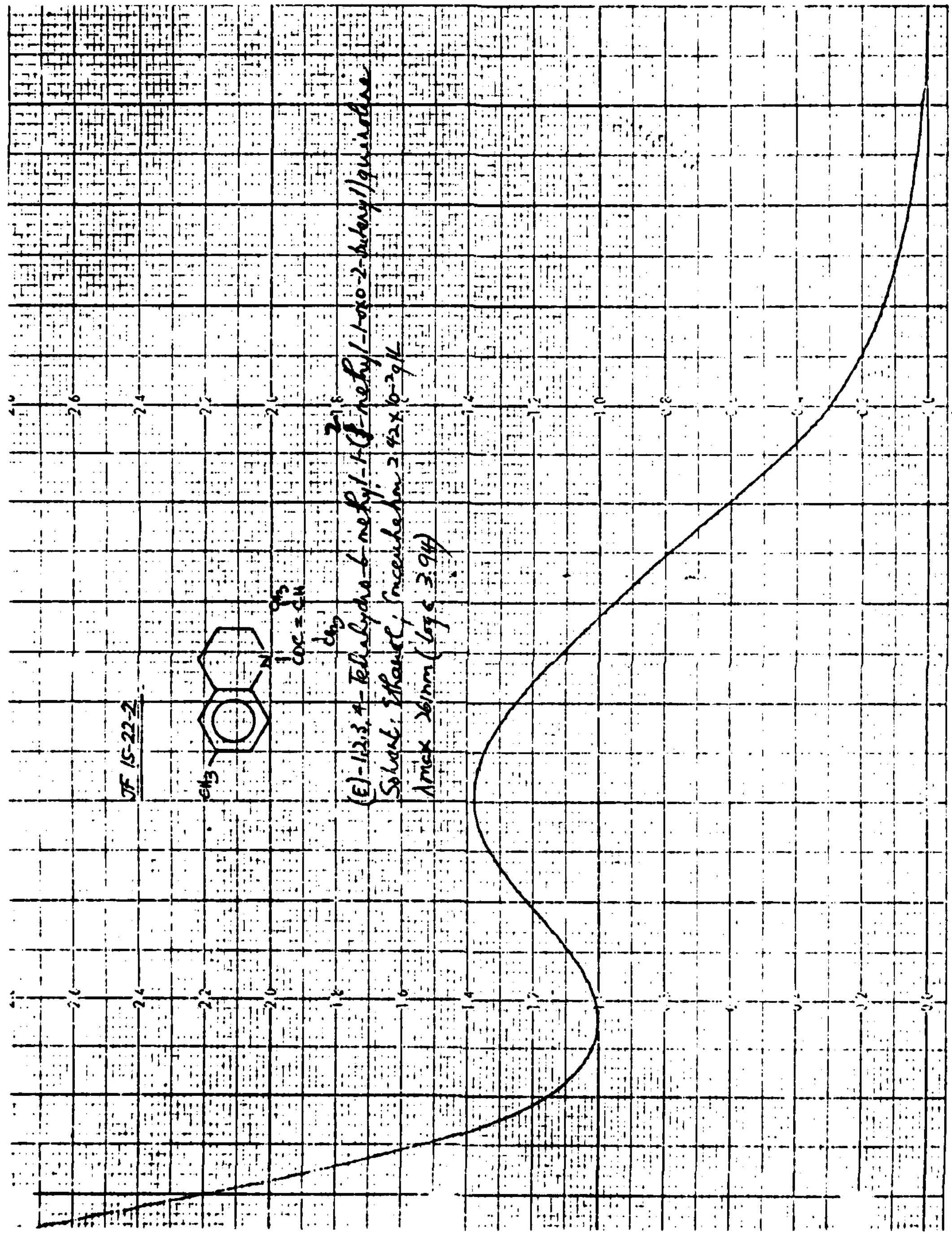
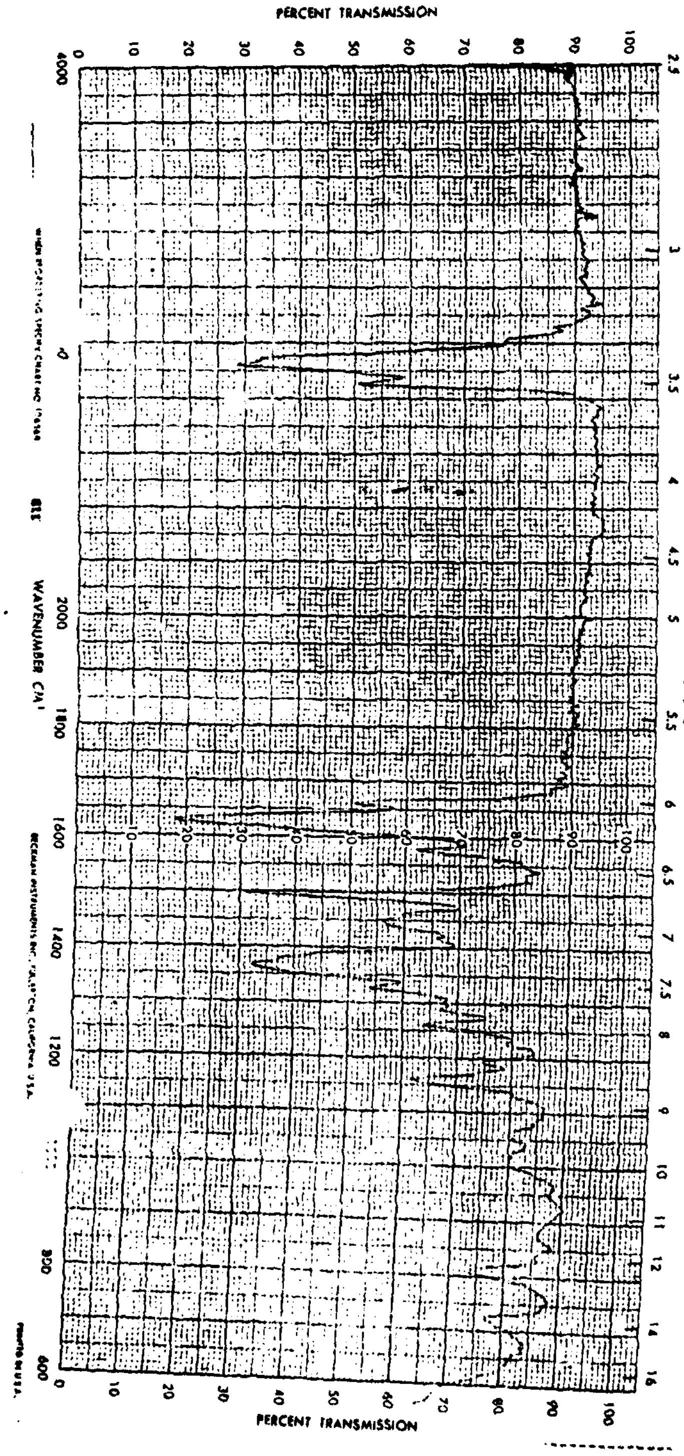
2/5/82


JF 15-22-2
(E)-1,2,3,4-Tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline
Solvent: Ethanol; Concentration 2.42x10-2 g/L
λmax 261nm (log ε 3.94)


PERCENT TRANSMISSION
WAVELENGTH IN MICRONS
WAVENUMBER CM⁻¹
PERCENT TRANSMISSION

LIST OF TABLES

APPENDIX B

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	14 mm	NG	+
1537	NG	16 mm	NG	15 mm	NG	+
WT	NG	14 mm	G	G	NG	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) CHR-5 - NG (b) NA (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 91, 106, 97 average = 98

(1) + = expected response - = unexpected response

Study Number: 82012 Date: 21 Apr 82 By: Sauers

TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: CHR 5 Substance dissolved in: ETOH

Study Number: 82012 Date: 21 Apr 82 Performed by: Sauers, Dacey

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	51	56	53	53	SL
10^{-1} mg/plate	90	73	81	81	NL
10^{-2} mg/plate	96	93	86	92	NL
10^{-3} mg/plate	87	61	107	85	NL
10^{-4} mg/plate	81	61	80	74	NL
10^{-5} mg/plate	120	99	93	104	NL
10^{-6} mg/plate	74	65	78	72	NL
10^{-7} mg/plate	73	71	82	75	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	NG (14 mm)	NG	+
100	NG	G	NG	NG (15 mm)	NG	+
1535	NG	NA	NG	NG (14 mm)	NG	+
1537	NG	NG	NG	NG (13 mm)	NG	+
1538	NG	NA	NG	NG (14 mm)	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) CHR5 - NG (b) NA (c) NA (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 82012 By: Sauers

(1) + = expected response

- = unexpected response

Date: 23 April 82

TABLE 4

SFONTANEOUS REVERTANT RATE AND POSITIVE CONTROL REVERTANT RATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
AF	2 ug/plate	yes	(703,865,697) 755	(619,545,533) 566			(652,933,959) 848
BF	2 ug/plate	yes	(67, 85, 91) 81	(498,456,489) 481		(51, 64, 65) 60	(76,108,106) 97
DMBA	20 ug/plate	yes	(999,999,999) 999	(999,999,999) 999		(28.,157,294) 247	(999,999,999) 999
MNNG	2 ug/plate	no		(999,999,999) 999			
	20 ug/plate	no			(999,999,999) 999		

Strain Ferformance

	98	100	1535	1537	1538
Spontaneous Revertants					
before after	(21, 23, 17) (16, 18, 20) 19	(121,113,139) (88,116,114) 115	(18, 26, 20) (13, 13, 22) 19	(5, 6, 4) (4, 5, 8) 5	(28, 16, 18) (16, 17, 21) 19
before after	(27, 29, 38) (22, 29, 26) 29	(125,128,106) (135,138,108) 123	(16, 18, 14) (17, 19, 13) 16	(4, 5, 9) (5, 3, 7) 6	(24, 28, 16) (11, 17, 27) 21

Study Number: 82012

Date: 23 April 82 By: Sauers, Mauk, Lewis, Dacey

Note: 999 means $\geq$ 999

TABLE 5

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
CHR5	10^{-1} mg/pl	no	(24, 18, 29) 24	(97, 109, 97) 101	(12, 16, 17) 15	(5, 5, 5) 5	(10, 12, 10) 11
		yes	(29, 19, 29) 26	(132, 120, 122) 125	(15, 23, 14) 17	(4, 4, 3) 4	(21, 18, 15) 18
	2×10^{-2} mg/pl	no	(23, 21, 10) 18	(110, 117, 75) 101	(19, 18, 18) 18	(7, 3, 3) 4	(10, 22, 16) 16
		yes	(36, 38, 27) 34	(108, 106, 122) 112	(26, 15, 17) 19	(7, 2, 6) 5	(15, 33, 20) 23
	4×10^{-3} mg/pl	no	(15, 25, 25) 22	(133, 121, 123) 126	(34, 24, 24) 27	(2, 5, 8) 5	(23, 16, 17) 19
		yes	(26, 29, 27) 27	(108, 117, 96) 107	(32, 24, 28) 28	(4, 5, 7) 5	(32, 29, 23) 28
	8×10^{-4} mg/pl	no	(25, 25, 21) 24	(119, 129, 80) 109	(20, 23, 23) 22	(2, 5, 3) 3	(20, 11, 19) 17
		yes	(31, 38, 29) 33	(127, 112, 98) 112	(25, 26, 31) 27	(3, 6, 2) 4	(27, 23, 12) 21

- continued

Study No.: 82012 Date: 23 Apr 82 Performed by: Sauers, Mauk, Lewis, Dacey

TABLE 5, concluded

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
CHR5	1.6 x 10^{-4} mg/pl	no	(16, 24, 25) 22	(129,119,126) 125	(15, 18, 16) 16	(4, 2, 5) 4	(19, 12, 15) 15
		yes	(23, 24, 24) 24	(126,127,108) 120	(15, 20, 23) 19	(6, 4, 5) 5	(25, 15, 21) 20
	3.2 x 10^{-5} mg/pl	no	(22, 15, 20) 19	(111,119, 96) 109	(9, 20, 14) 14	(3, 3, 5) 4	(13, 11, 18) 14
		yes	(22, 25, 36) 28	(95,105,108) 103	(27, 21, 23) 24	(7, 2, 14) 8	(22, 19, 21) 21

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